輸血および血漿分画製剤使用同意書

東京医科大学八王子医療センター長　殿

私は輸血および血漿分画製剤の使用の必要性，副作用の可能性などについて，別紙の輸血および血漿分画製剤の使用説明書を基に下記の説明を受け，十分理解しました．治療または検査に必要と考えますので，輸血を受けることおよび血漿分画製剤の使用に同意します．

平成　　年　　月　　日　　患者氏名＿＿＿＿＿＿＿＿＿＿＿＿＿＿／サイン
代理人氏名＿＿＿＿＿＿＿＿＿＿＿＿＿＿／サイン

1. 私の治療または検査に際して，輸血または血漿分画製剤の使用が必要なこと，またその可能性があること．
   使用理由：出血，手術，貧血，血小板減少，凝固因子低下，循環血漿量低下，免疫グロブリン低下
   その他（　　）
2. 輸血および血漿分画製剤を使用しなかった場合，重篤な合併症を起こす危険性があること．
3. 予測される輸血および血漿分画製剤の使用量（説明者が具体的に記入すること）
   ＊担当医は可能性のあるすべての製剤の使用予定量をかならず記入して下さい．

輸血用血液製剤		血漿分画製剤	
種　類	使用予定量	種　類	使用予定量
濃厚赤血球	単位	アルブミン製剤	
新鮮凍結血漿	単位	免疫グロブリン製剤	
濃厚血小板	単位	血液凝固因子製剤	
自己血	単位	アンチトロンビンⅢ製剤	
		フィブリン糊製剤	
		その他（　　）	

4. 輸血および血漿分画製剤の安全性は格段に向上しているが，輸血による感染症には回避できないものもあること．
   また免疫副作用（輸血後 GVHD，溶血反応，発熱，じんま疹など）をきたす可能性があること．
5. 輸血には，1）献血による同種血輸血（他人の血液の輸血）と，2）自分の血液を輸血する自己血輸血があること．
   また自己血輸血の場合，準備した自己血が不足すれば，同種血輸血もありうること．
6. 輸血による感染の有無を確認するため，輸血前と輸血実施の2〜3カ月後に肝炎ウイルス検査とヒト免疫不全ウイルス（HIV）検査の実施が推奨されていること．また，輸血後の感染を確認するために，輸血前に保管用の血液を採取し一定期間保管すること．
7. 輸血および血漿分画製剤の使用記録を定められた期間保存し，必要のある時には輸血用血液および血漿分画製剤の製造者または厚生労働大臣へこの記録を提供すること．
8. 血漿分画製剤には，人の血漿から製造したものと，遺伝子組み換え技術により製造した同じ効果を有する製品があること．
   また遺伝子組み換え技術により製造された製品の一部は，使用前にアレルギー検査が必要なこと．
9. 血漿分画製剤の原料血漿には，献血由来と非献血由来があること．
10. 輸血や血漿分画製剤の使用により感染症にかかり健康被害を受けた方の救済を図るための生物由来製品感染等被害救済制度があること．
11. 安全で高度な輸血医療を提供するために，追加検査（まれな血液型に関する検査，副作用の原因調査や防止に関する検査，感染症に関する検査など）を実施する場合があること．

図2　輸血および血漿分画成分輸注に関する同意書の一例

て，定期的に患者説明の内容を検証し，更新してゆく必要がある．患者の理解度を把握するためのアンケート調査を行い，説明医へフィードバックしたり，患者の希望を反映できるような体制の整備が求められる．

文献
1）厚生労働省編：血液製剤の使用にあたって（第3版）．じほう，2005.
2）森岡恭彦：インフォームドコンセント．日本医師会雑誌，**135**（7）：1511〜1514，2006.

Japanese Journal of Transfusion and Cell Therapy, Vol. 57. No. 3　57(3)：178—183, 2011

—[報　告]— Report —

パイロット研究による輸血副作用の解析
—我国における包括的なヘモビジランスの構築に向けて—

加藤　栄史[1)]　高本　滋[1)]　小高千加子[2)]　佐川　公矯[3)]　星　順隆[4)]
藤井　康彦[5)]　米村　雄士[6)]　岩尾　憲明[7)]　田中　朝志[8)]　岡崎　仁[9)]
百瀬　俊也[9)]　北澤　淳一[10)]　森　宏[11)]　松下　明夫[12)]　野村　久子[13)]
八十嶋　仁[14)]　大日　康史[15)]　岡田　義昭[2)]　浜口　功[2)]　山口　一成[2)]

輸血療法は有効かつ必須の治療法であるが，血液製剤は他人の血液を原料とするため，輸血副作用を完全には回避できない．このため，輸血副作用に関する可及的速やかな実態把握とその対策が必要と考えられる．本研究ではオンラインによる副作用報告システムのパイロット研究を開始し，収集したデータを解析，検討した．2007年より7大学病院を対象に本研究を開始し，2009年から小規模（300床以下）の5施設を加えて，輸血量，副作用件数を2カ月間隔で収集した．尚，本研究では2007年1月から3年間の結果を報告する．

輸血副作用の発生率はバッグ当り1.50％であり，我が国の輸血副作用発生頻度を反映していると考えられた．また，血小板製剤（PC）の副作用発生率が4.34％と他の2製剤に比して約6倍の高頻度であり，一因として頻回輸血に伴う同種抗体の産生など免疫学的機序が関与することが推察された．一方，各施設における診療疾患の相違により副作用発生頻度に差異が認められた．

本研究で構築したシステムは，輸血副作用の現状を正確に把握するために有用であり，広く普及させることで，よりよいヘモビジランスの構築に貢献できると考えられる．

キーワード：ヘモビジランス，輸血副作用，オンライン報告，パイロット研究，発生率

はじめに

輸血療法は極めて有効かつ必須の治療法である．しかしながら，血液製剤は他人の血液を原料とするため，感染症や免疫反応などの輸血副作用を完全には回避しきれない．我が国では核酸増幅検査(Nucleic-acid Amplification Test：NAT)の導入により輸血感染症に対する安全性は向上し，致命率の高い輸血後GVHD(Graft versus Host disease)は放射線照射の普及により激減した．しかしながら，輸血過誤や輸血副作用の大多数を占める免疫学的副作用の発生頻度については著明な減

1）愛知医科大学輸血部
2）国立感染症研究所血液・安全性研究部
3）久留米大学臨床検査部
4）東京慈恵会医科大学輸血部
5）山口大学輸血部
6）熊本大学輸血・細胞治療部
7）山梨大学輸血細胞治療部
8）東京医科大学八王子医療センター
9）日本赤十字社血液事業本部
10）黒石市国民健康保険黒石病院輸血療法管理室
11）国民健康保険南多摩病院
12）士別市立病院
13）東京都教職員互助会三楽病院
14）八尾総合病院
15）国立感染症研究所感染症情報センター
〔受付日：2010年10月14日，受理日：2011年3月10日〕

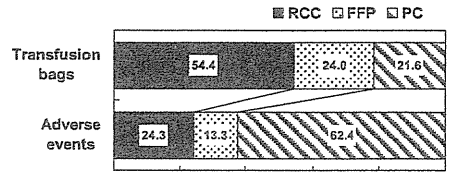
少が認められず，近年では輸血関連急性肺障害(Transfusion Related Acute Lung Injury：TRALI)[1]や輸血関連循環過負荷（Transfusion-Associated Circulatory Overload：TACO)[2]などの重篤な副作用も認められている．このため，輸血副作用に関する可及的速やかな実態把握とその対策が必要と考えられる．

輸血副作用の実態把握に関してはエイズ感染以降，欧州では輸血副作用を監視するヘモビジランス(hemovigilance)という体制が確立されている．我が国では日本赤十字社が輸血副作用の収集を行っているが，重症副作用症例の原因検索依頼を兼ねた医療施設からの自発報告が中心であり，重症に偏りがちであり[3]，必ずしも輸血副作用全体が把握されているとは言い難い．我が国の輸血副作用を厳密に把握するには，全国的な副作用の報告体制を確立することが必須と考えられる．我々は，簡便かつ迅速に全ての副作用を収集する体制を確立する目的で，国立感染症研究所を副作用の収集・解析センターとし，2007年11月よりインターネットを利用したオンラインによる輸血副作用報告システムのパイロット研究を開始した．今回，我々は，このパイロット研究で収集した副作用について解析，検討を行ったので報告する．

方　法

愛知医科大学病院，熊本大学病院，久留米大学病院，東京医科大学八王子医療センター，東京慈恵会医科大学病院，山口大学病院，山梨大学病院の7大学病院を対象に2007年11月よりインターネットを利用したオンラインによる輸血副作用報告システムのパイロット研究を開始した．データの収集，解析は国立感染症研究所および愛知医科大学病院にて実施した．さらに，2009年1月より病床数が300床以下の5施設（市立士別総合病院，黒石市国民健康保険黒石病院，東京都国民健康保険団体連合会南多摩病院，東京都教職員互助会三楽病院，医療法人医真会八尾総合病院）が新たに参加した．各施設からの報告は2カ月ごとにオンラインにて行われた．報告内容は輸血用血液製剤別の使用単位数および使用バッグ数，さらに，「免疫学的輸血副作用の把握とその対応に関する研究」研究班（H17-医薬一一般-053，高本班)[4]が作成した副作用の症状項目，診断項目表を用いた症状別ならびに診断別の件数である．ただし，副作用件数での入力であることから，現在，入力の暗号化，匿名化は実施していない．また，本研究では2007年1月から2009年12月までの3年間について解析した．

A.



B.

	RCC	FFP	PC	Total
No. of transfusion bags	95,963	42,127	38,141	176,231
No. of adverse events	644	351	1,654	2,649
Incidence (%)	0.64	0.83	4.34	1.50

Fig. 1　Ratio of transfusion bags and adverse events according to blood component. (A) Ratio of transfusion bags (upper) and adverse events (lower) according to blood component. (B) Number of transfusion bags and adverse events, and incidence according to blood component. RCC; red cell concentrate, FFP; fresh frozen plasma, PC; platelet concentrate

結　果

1. 3年間における輸血副作用の発生状況

本研究が開始された2007年から2009年までの3年間で輸血された総バッグ数は176,231バッグであった．その内，赤血球製剤（RCC）は95,963バッグで全体の約54%を占め，新鮮凍結血漿(FFP)が42,127バッグ，血小板製剤(PC)が38,141バッグとそれぞれ24%，22%を占めていた(Fig. 1A)．一方，輸血副作用に関しては，総件数が2,649件であり，バッグ当り1.50%の頻度であった．副作用の原因となった製剤別の割合を見ると，PCが62.4%と半数以上を占め，残りをRCCが24.3%，FFPが13.3%を占めていた．使用製剤別の割合と比較すると，PCが約3倍に増加し，その分，RCCおよびFFPの占める割合が減少した．また，バッグ当りの副作用発生率はRCCやFFPでは0.64%，0.83%と1%未満であったのに対し，PCは4.34%と約6倍の高頻度であった（Fig. 1B)．

副作用の種類について，RCCでは発熱反応が約40%，アレルギー反応が約30%であり，残りは呼吸器症状，血圧上昇，血圧低下などであった．また，FFPやPCではアレルギー反応が各々69%，79%と大半を占め，発熱反応が各々13%，12%であった(Fig. 2A)．一方，バッグ当りの副作用発生率を症状別に見ると，RCCでは，発熱が0.22%，蕁麻疹が0.16%と多く，FFPでは，掻痒感や蕁麻疹が各々0.20%，0.48%であり，PCでは，発熱，掻痒感，蕁麻疹が各々0.36%，0.98%，3.00%であった（Fig. 2B)．

2. 輸血副作用の年次推移

各年次について，輸血副作用発生率の推移を見るため，本研究開始時からの対象施設である7大学病院に限定して，発生率を検討した．その結果，Fig. 3Aに示

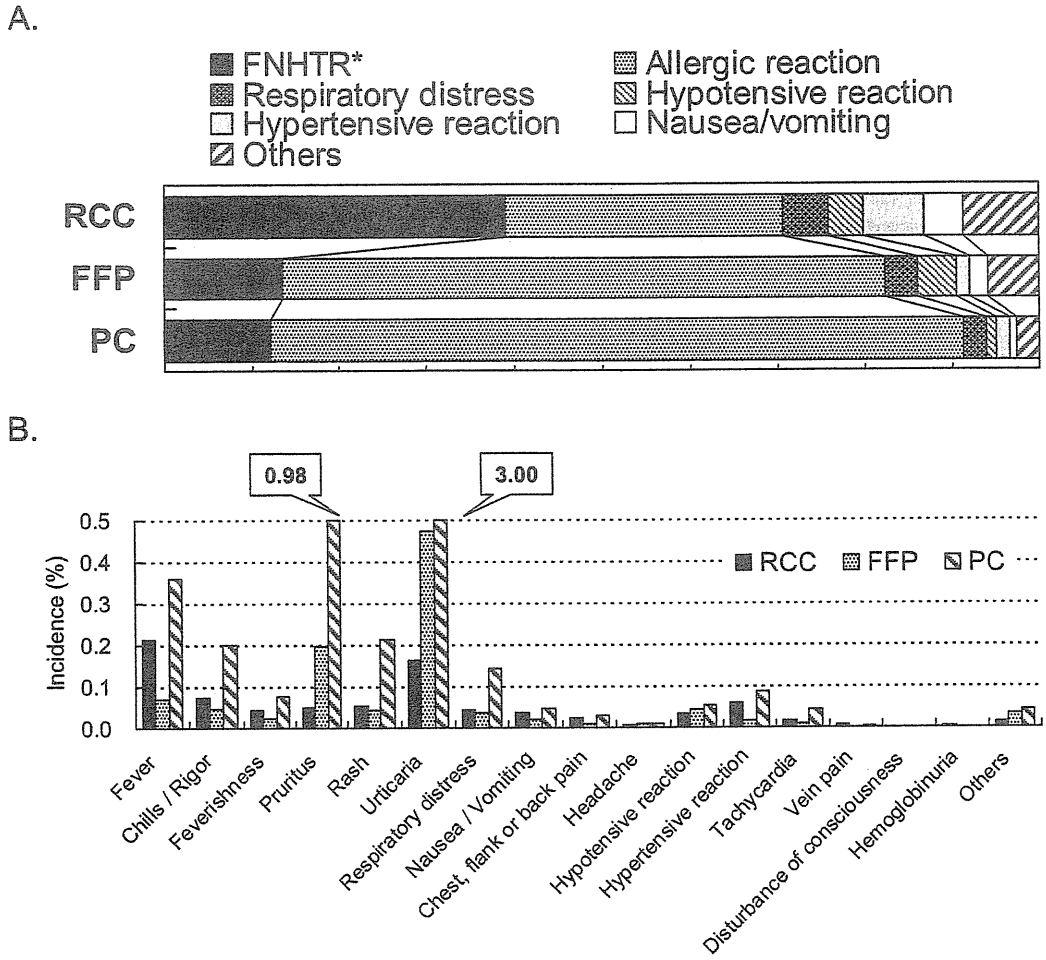


Fig. 2 Types of adverse event (A) and incidence (B) by blood component. (A) Distribution of types of adverse event by blood component. (B) Incidence of types of adverse event per bag. FNHTR; febrile non-hemolytic transfusion reaction

A.

Year	RCC (%)	FFP (%)	PC (%)	Total (%)
2007	0.54	0.63	3.44	1.16
2008	0.61	0.69	4.22*	1.45*
2009	0.79**	1.19**	5.36**	1.91**

B.

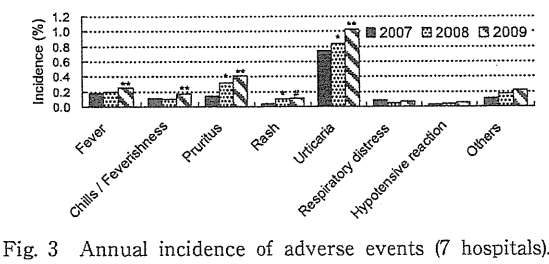


Fig. 3 Annual incidence of adverse events (7 hospitals). (A) Annual incidence of adverse events. (B) Annual incidence of types of adverse events. *: $p<0.01$ when compared with those in 2007, **: $p<0.01$ when compared with those in 2007 and 2008

すごとく，バッグ当りの副作用発生率では年次ごとに有意な上昇が認められた．製剤別で見ると，RCC および FFP では 2009 年次が過去 2 年次に比較し，また，PC では，年次ごとに有意な上昇が認められた．

次に，副作用の発生率が上昇した要因の症状を検討した．その結果，Fig. 3B に示すごとく，副作用の多くを占める発熱，悪寒・戦慄などの発熱反応や，掻痒感，発赤，蕁麻疹などのアレルギー反応の発生率が年次により有意な上昇を示した．

3. 輸血副作用発生率の施設間差

本研究に参加している 12 施設間の差を検討するため，全参加施設からのデータがそろう 2009 年次に限定して，各施設での副作用発生率の比較検討を行った．その結果，バッグ当りの総副作用発生率を見ると，0% から 3.67% と大きな差が認められた（データ非提示）．

さらに，12 施設を病床数が 500 床以上で，PC を頻回に輸血する血液疾患などを診療している大規模病院 7 施設（大規模群）と 300 床以下で，血液疾患など特殊

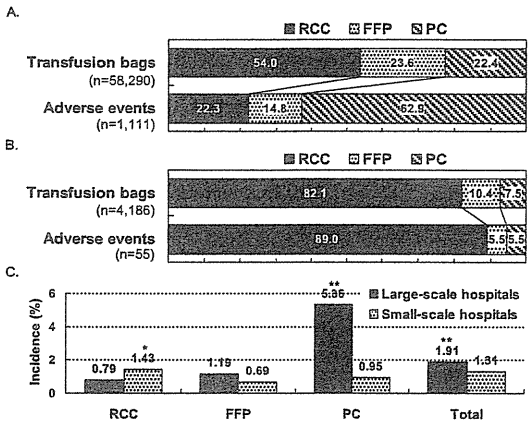


Fig. 4　Comparison of transfusion bags and adverse events, and incidence between large- and small-scale hospitals in 2009. (A) Ratio of transfusion bags and adverse events according to blood component in large-scale hospitals (7 hospitals). (B) Ratio in small-scale hospitals (5 hospitals). (C) Incidence of adverse events per bag in large- and small-scale hospitals. *: $p<0.01$ compared with large-scale hospitals, **: $p<0.01$ compared with small-scale hospitals

な疾患を扱っていない小規模病院5施設(小規模群)に大別して,2群間における輸血状況と副作用発生率の比較を行った．その結果，Fig. 4A，Bに示すごとく，大規模群では,使用輸血バッグ数の内訳でRCCが約半数，残りをFFPとPCが占め，副作用でPCが約6割，残りをRCCとFFPが占めていた．一方，小規模群では，使用輸血バッグ数や副作用の内訳でRCCが8割以上を占めるのに対し，PCは各々7.5%，5.5%を占めるに留まり，大規模群に比して割合が低率であった．また，Fig. 4Cに示すごとく,バッグ当りの副作用発生率については,大規模群が1.91%と小規模群の1.31%に比して有意に高率であった．さらに，製剤別では，RCCが大規模群の0.79%に対して小規模群が1.43%と有意に高率であり，PCでは大規模群が5.36%と小規模群(0.95%)の約5倍強と高率であった．この様に，血液製剤の使用内容の相違により副作用の発生率が異なることが認められた．

考　察

本研究で解析対象とした輸血用血液製剤量は3年間で176,231バッグであり,1年間での平均使用血液製剤量は58,744バッグと2008年度に日本赤十字社が全国の医療機関に供給した血液製剤量[3]の1.20%に相当した．また，製剤別に関しても，各供給量のRCCが0.99%，FFPが1.51%,PCが1.75%に相当し,本調査結果が全国の輸血使用量の約1%に基づく結果と見做される．

3年間の総輸血副作用件数は2,649件であり,副作用発生率はバッグ当り1.50%であった．ただし，欧州からの報告[5)~8)]では,副作用発生率が1,000バッグ当り2.2～4.2と本研究の結果より低頻度であった．この相違の一因として，発生した症状・所見と輸血との関連性について不明確で，輸血によらない副作用が含まれている可能性があり，今後の検討課題と考えられる．一方，臨床現場において,「輸血療法の実施に関する指針」[9]が遵守され，輸血中および輸血後の患者観察が厳密に実施され，軽症の副作用も漏れなく把握されていることによるとも推察される．事実，本研究の副作用発生頻度は輸血副作用把握に積極的と評価されている特定施設を対象とした厚生労働省「輸血副作用把握体制の確立」研究班（H17-医薬一一般-053，高本班)[10]からの報告と同頻度であった．これらのことから，本研究の発生頻度は，日本における輸血副作用発生率を反映していると考えられた．また，これまで，日本赤十字社や医療機関で輸血副作用に対して予防策が講じられているものの，バッグ当り約1%強の頻度で副作用が発生することが示され，欧州に比べ高頻度であることからも，輸血副作用軽減のため更なる対策が必要と考えられる．

次に，血液製剤別の副作用発生頻度については，PCが4.34%とRCC,FFPに比して約6倍の高頻度であった(Fig. 1B)．同様の結果が日本赤十字社[11]，欧州[5]からも報告されている．また，RCCでの副作用は発熱や悪寒を中心とした発熱反応の割合が多く，血漿成分が主体であるFFPやPCでの副作用は蕁麻疹や掻痒感などのアレルギー反応が中心であった．発熱反応は主として血液製剤中に混入している白血球から産生されるサイトカインなど，アレルギー反応は血漿中に存在する様々なタンパク，抗体などに起因すると考えられている[12]．PCは血液疾患に対して使用される場合が多く，しかも繰り返し,頻回に投与される場合が殆どである．本研究でも，血液疾患を積極的に治療している大規模群は血液疾患を診療していない小規模群に比してPCの輸血量が多く，副作用発生率が有意に高率であった(Fig. 4)．事実，安藤ら[13]は頻回輸血患者に副作用の発生率が高い事を報告している．このことから,PCが他の製剤に比して副作用が高頻度である一因として，頻回輸血に伴う同種抗体の産生など免疫学的機序が関与することが推察される．

輸血副作用を正確に把握するには，副作用の報告体制を確立するとともに，輸血に関わる医師，看護師，検査技師の輸血副作用に対する認識，理解，協力が必須である．本研究では，簡便かつ容易であり，有用性の評価された「輸血副作用の症状項目」や「副作用症状からの診断項目表」[4]を用いることにより，副作用報告の統一性が向上し，臨床現場での副作用に対する認識度が高くなってきたものと考えられる．事実，本研

究の開始時からの年次推移を見ると，診療内容ならびに血液製剤の内容について大きな変化がないにも拘わらず，副作用発生率が徐々に上昇を示していた（Fig. 3）．また，これまでの副作用頻度は大規模施設を対象とした報告[14)~16)]であり，各施設での診療疾患がほぼ同様であることから，施設間での大きな相違が認められなかった．ただし，前述した様に，各施設における血液疾患の診療の有無など診療疾患の相違により製剤別の副作用頻度が異なることが示された．

輸血副作用の診断に関しては，複数製剤輸血時における起因性の判断，TRALI や TACO などの鑑別診断など，解決すべき課題があるものの，本研究で構築した輸血副作用のオンライン報告システムは，各施設における副作用に対する認識を向上させ，現時点における副作用実態を迅速に把握することを可能にさせる有用な手段と考えられる．さらに，各血液製剤のリスクや診療疾患による副作用頻度の相違，また，これまで導入された副作用防止策の評価などを明らかにすることのできる重要なシステムと考えられる．今後，本研究を基に全国的なヘモビジランスを確立することにより，安全な輸血医療の確立に資することができるものと期待される．

謝辞：本研究で，輸血副作用の収集・解析にご協力頂いた国立感染症研究所血液・安全性研究部の大隈和先生，種市麻衣子先生に深謝いたします．

文　献

1) Bux J: Transfusion-related acute lung injury (TRALI): a serious adverse event of blood transfusion. Vox Sang, 89: 1—10, 2005.
2) Rana R, Fernandez-Perez ER, Khan SA, et al: Transfusion related acute lung injury and pulmonary edema in critically ill patients: a retrospective study. Transfusion, 46: 1478—1483, 2006.
3) 日本赤十字社血液事業部医薬情報課：赤十字血液センターに報告された非溶血性輸血副作用—2008 年—, 2009.
4) 高本　滋，加藤栄史，宇留間元昭，他：輸血副作用の症状および診断項目表作成と輸血副作用の実態調査，厚生労働省科学研究費補助金「免疫学的輸血副作用の把握とその対応に関する研究」総合研究報告書，2008, 17—37.
5) Siegenthaler MA, Schneider P, Vu DH, et al: Haemovigilance in a general university hospital: need for a more comprehensive classification and a codification of transfusion-related events. Vox Sang, 88: 22—30, 2005.
6) Michlig C, Vu DH, Wasserfallen JB, et al: Three years of haemovigilance in a general university hospital. Transfusion Medicine, 13: 63—73, 2003.
7) Rouger P, Noizat-Pirenne F, Le Pennec PY: Haemovigilance and transfusion safety in France. Vox Sang, 78 (Suppl. 2): 287—289, 2000.
8) Andreu G, Morel P, Forestier F, et al: Hemovigilance network in France: organization and analysis of immediate transfusion incident reports from 1994 to 1998. Transfusion, 42: 1356—1364, 2002.
9) 厚生労働省編：血液製剤の使用にあたって第 3 版—輸血療法の実施に関する指針，じほう社，東京，2005.
10) 高本　滋，加藤栄史，宇留間元昭，他：特定施設における輸血副作用の実態調査，厚生労働省科学研究費補助金「輸血副作用把握体制の確立—特に免疫学的副作用の実態把握とその対応—」平成 21 年度報告書，2010, 18—33.
11) 村岡正人，相坂直子，百瀬俊也，他：日本赤十字社に報告された非溶血性副作用の現状—2006 年—．日本輸血細胞治療学会誌，55：500—507, 2009.
12) Enright H, Davis K, Gernsheimer T, et al: Factors influencing moderate to severe reactions to PLT transfusions: experience of the TRAP multicenter clinical trial. Transfusion, 43: 1545—1552, 2003.
13) 安藤高宣，加藤井久子，林　恵美，他：実患者数に基づいた輸血副作用の頻度．日本輸血学会誌，49：327, 2003.
14) 藤井康彦，浅井隆善，下平滋隆，他：重篤な急性輸血副作用に関する多施設共同研究．日本輸血細胞治療学会誌，54：406—410, 2008.
15) 倉田義之，清水　勝，岡崎　仁，他：免疫学的機序による非溶血性輸血副作用頻度実態調査報告．日本輸血細胞治療学会誌，53：43—46, 2007.
16) 下平滋隆，藤井康彦，桝屋正浩，他：全国国立大学附属病院における輸血副作用調査体制—輸血副作用の原因製剤回収・保管について調査報告—．日本輸血細胞治療学会誌，52：711—716, 2006.

ANALYSIS OF TRANSFUSION-RELATED ADVERSE EVENTS BASED ON A PILOT STUDY —TOWARD A COMPREHENSIVE HEMOVIGILANCE SYSTEM FOR JAPAN—

Hidefumi Kato[1], *Shigeru Takamoto*[1], *Chikako Odaka*[2], *Kimitaka Sagawa*[3], *Yasutaka Hoshi*[4], *Yasuhiko Fujii*[5], *Yuji Yonemura*[6], *Noriaki Iwao*[7], *Asashi Tanaka*[8], *Hitoshi Okazaki*[9], *Shun-ya Momose*[9], *Junichi Kitazawa*[10], *Hiroshi Mori*[11], *Akio Matsushita*[12], *Hisako Nomura*[13], *Hitoshi Yasoshima*[14], *Yasushi Ookusa*[15], *Yoshiaki Okada*[2], *Isao Hamaguchi*[2] *and Kazunari Yamaguchi*[2]

[1]Department of Transfusion Medicine, Aichi Medical University

[2]Department of Safety Research on Blood and Biological Products, National Institute of Infectious Diseases

[3]Department of Laboratory Medicine, Kurume University

[4]Department of Blood Transfusion, Tokyo Jikeikai Medical University

[5]Department of Blood Transfusion, Yamaguchi University School of Medicine

[6]Department of Transfusion Medicine and Cell Therapy, Kumamoto University

[7]Division of Transfusion Medicine and Cell therapy, University of Yamanashi

[8]Department of Blood Transfusion, Tokyo Medical University Hachioji Medical Center

[9]Central Blood Institute, Blood Service Headquarters, Japanese Red Cross Society

[10]Division of Transfusion, Kuroishi General Hospital

[11]Minami Tama Hospital

[12]Shibetsu City Hospital

[13]Sanraku Hospital

[14]Yao General Hospital

[15]Infectious Disease Surveillance Center, National Institute of Infectious Diseases

Abstract:

Although blood transfusion is an essential and effective therapy, transfusion-related adverse events remain possible due to the allogeneic origin of blood products. In order to grasp and deal with these adverse events, a comprehensive reporting system, namely a hemovigilance system, needs to be established in Japan. We have been running a pilot study since 2007, in which all grades of transfusion-related adverse events are collected bimonthly using an online system. Seven university hospitals initially participated in this study, and were joined by five small hospitals (with fewer than 300 beds) in 2009. This paper reports the result of analysis of the data collected from 2007 to 2009.

Over the three years, the overall incidence of adverse events per bag was 1.50%. Platelet concentrate (PC) (4.34%) gave rise to statistically more adverse events (6-fold) than both red cell concentrate and fresh frozen plasma. This difference was possibly caused by allo-immunization of PC recipients, because these patients, who commonly have with hematological diseases, tend to receive regular blood transfusions, and thus to be frequently allo-immunized. In addition, we found that the incidence of adverse events varied between hospitals by number of beds and patient characteristics.

In conclusion, this online reporting system may be useful for the collection and analysis of actual adverse events in recipients, and may contribute to the development of a more comprehensive hemovigilance system in Japan.

Keywords:

Hemovigilance, Transfusion-related adverse events, Online report, Pilot study, Incidence

Journal Web Site: http://www.jstmct.or.jp/jstmct/

Int J Hematol (2011) 94:71–80
DOI 10.1007/s12185-011-0886-8

ORIGINAL ARTICLE

Romiplostim for the treatment of chronic immune thrombocytopenia in adult Japanese patients: a double-blind, randomized Phase III clinical trial

Yukari Shirasugi · Kiyoshi Ando · Koji Miyazaki · Yoshiaki Tomiyama · Shinichiro Okamoto · Mineo Kurokawa · Keita Kirito · Yuji Yonemura · Shinichiro Mori · Kensuke Usuki · Koji Iwato · Satoshi Hashino · Helen Wei · Richard Lizambri

Received: 27 April 2011 / Revised: 1 June 2011 / Accepted: 1 June 2011 / Published online: 25 June 2011

Abstract The efficacy and safety of romiplostim, a thrombopoietin-mimetic peptibody, were evaluated in a double-blind, placebo-controlled, randomized trial of Japanese patients with chronic immune thrombocytopenia (ITP). Thirty-four ITP patients received romiplostim ($n = 22$) or placebo ($n = 12$) for 12 weeks, with a starting romiplostim dose of 3 μg/kg weekly. The primary end point was the number of weeks with platelet response, defined as a platelet count $\geq 50 \times 10^9$/L (not including the 4 weeks after rescue medication administration). Patients received a median of 4 (range 1–19) prior ITP therapies including splenectomy in 44%. On study, 68% also received concomitant ITP therapy. Weekly responses occurred for a median of 11 weeks with romiplostim as compared to 0 weeks with placebo ($p < 0.0001$). Most romiplostim-treated patients (95%) achieved platelet responses; two showed extended responses after the treatment period. The use of rescue medication was required in

The work was done at multiple clinical investigational sites within Japan.

Y. Shirasugi (✉) · K. Ando
Department of Hematology and Oncology,
Tokai University Hospital, 143 Shimokasuya,
Isehara, Kanagawa 259-1193, Japan
e-mail: whiteced@is.icc.u-tokai.ac.jp

K. Miyazaki
Department of Hematology, Kitasato University Hospital,
Kanagawa, Japan

Y. Tomiyama
Department of Blood Transfusion,
Osaka University Hospital, Osaka, Japan

S. Okamoto
Division of Hematology, Keio University Hospital,
Tokyo, Japan

M. Kurokawa
Department of Hematology and Oncology,
The University of Tokyo Hospital, Tokyo, Japan

K. Kirito
Department of Hematology and Oncology,
University of Yamanashi Hospital, Yamanashi, Japan

Y. Yonemura
Department of Transfusion Medicine and Cell Therapy,
Kumamoto University Hospital, Kumamoto, Japan

S. Mori
Department of Hematology and Oncology,
Kansai Medical University Hirakata Hospital, Osaka, Japan

K. Usuki
Division of Hematology, NTT Kanto Medical Center,
Tokyo, Japan

K. Iwato
Department of Blood Transfusion, Hiroshima Red Cross
Hospital and Atomic-bomb Survivors Hospital,
Hiroshima, Japan

S. Hashino
Department of Gastroenterology and Hematology,
Hokkaido University Hospital, Hokkaido, Japan

H. Wei
Biostatistics, Amgen Inc., Thousand Oaks, CA, USA

R. Lizambri
Clinical Development, Amgen Inc., Thousand Oaks, CA, USA

9% of romiplostim-treated patients as compared with 17% of placebo-treated patients. Both treatment groups had similar incidences of adverse events (91% romiplostim, 92% placebo). Adverse events that occurred more frequently (>10%) in romiplostim-treated patients included nasopharyngitis, headache, peripheral edema, back pain, and extremity pain. In conclusion, romiplostim significantly increased and maintained platelet counts and was well tolerated in Japanese patients with ITP.

Keywords Idiopathic thrombocytopenic purpura · TPO-receptor agonist · Japan · Efficacy · Safety

1 Introduction

Immune thrombocytopenia (ITP) is an immune-mediated disorder characterized by low peripheral platelet counts (i.e., $<100 \times 10^9$/L) due to both increased platelet destruction and impaired platelet production [1–8]. A diagnosis of primary ITP is made based on history, complete blood counts, and exclusion of potential underlying causes [9]. ITP in adults generally appears in a chronic form, with the definition of chronic ranging from ITP of at least 6 months duration to at least 12 months duration [7, 9–12]. Causal factors may include active *Helicobacter pylori* infection; *H. pylori* eradication therapy appears to improve thrombocytopenia in some ITP patients [13–18]. ITP appears to be more frequent in women, particularly between the ages of 30–60 years [10, 11, 19, 20]. Recent reports of incidence rates range from 1.6 to 3.9 per 100,000 person-years in Northern European populations [10, 19, 20], and both incidence and prevalence of chronic ITP increase with age [10, 19, 20]. The incidence and prevalence of ITP in Japan is similar to that observed in other countries, with approximately 70% of cases occurring in patients older than 50 years [15, 21].

As the clinical presentation of ITP varies considerably, the management of chronic ITP is best tailored to the individual patient [5, 9]. In Japan, *H. pylori* eradication therapy is recommended as first-line therapy for *H. pylori*-positive ITP patients [15]. Treatments for ITP are only clearly indicated for those patients with an extremely low platelet count ($<10 \times 10^9$/L) and/or with severe bleeding symptoms [22]. Treatment is not recommended for patients with platelet counts $>50 \times 10^9$/L in the absence of bleeding, trauma/surgery, or other risk factors [11]. Available treatment options include corticosteroids and other immunosuppressive agents, splenectomy, and immunoglobulins [6, 11, 23]. Approximately, 20–35% of ITP patients have disease refractory to treatment with steroids, immunoglobulins, or splenectomy [22, 24], and only two-thirds of patients undergoing splenectomy experience sustained response without additional therapy [11]. As not all patients respond to these treatment options, which are often associated with various side effects [22], additional safe and effective management strategies are needed.

Most current therapies aim to reduce platelet destruction. Treatments aimed at increasing thrombopoiesis, such as the thrombopoietin (TPO)-receptor agonists romiplostim and eltrombopag, offer a potentially complementary treatment strategy for ITP [25, 26]. These second-generation thrombopoietic agents are recommended for chronic ITP in patients failing initial therapy with steroids/immunoglobulins or relapse after splenectomy [11]. Romiplostim is a subcutaneously administered F_c-peptide fusion protein composed of 2 identical peptide subunits that activate the TPO receptor, thus leading to an increase in megakaryopoiesis [27]. In studies outside of Japan, romiplostim was shown to be effective for the treatment of chronic ITP with good tolerability. Romiplostim is approved in the USA for the treatment of chronic ITP that has not responded to corticosteroids, immunoglobulins, or splenectomy, and in Europe for the treatment of splenectomized patients with chronic ITP refractory to other treatments or in non-splenectomized patients in whom splenectomy is contraindicated [27]. As of January 2011, romiplostim was approved by the Japanese regulatory authority Pharmaceuticals and Medical Devices Agency (PMDA) for the treatment of chronic ITP [28].

Racial groups may differ in terms of disease presentation, adverse-event profiles, drug metabolism, and response to treatment [29, 30]. The potential for clinically relevant efficacy and safety differences between Japanese and Western populations treated with romiplostim thus required assessment. Romiplostim was previously assessed in phase 1 and 2 dose-escalation studies in Japanese patients with ITP. In these studies, romiplostim was found to be safe, well tolerated, and effective at increasing platelet counts in a dose-dependent manner [31, 32]. Results were generally consistent with those from studies on non-Japanese patients. Data from phase 2 studies suggested a romiplostim starting dose of 3 μg/kg in Japanese patients. To further investigate the use of romiplostim in this setting, we conducted a randomized, blinded, phase 3 trial evaluating the efficacy and safety of romiplostim (starting dose of 3 μg/kg) compared with placebo as second-line therapy for chronic ITP in adult Japanese patients.

2 Materials and methods

2.1 Study design and patients

This was a randomized, placebo-controlled, double-blind phase 3 study, similar in design to previous global

romiplostim trials [33, 34]. The study was conducted between 20 November 2007 and 13 April 2009 and enrolled ITP patients from 11 study centers in Japan. The trial adhered to Japanese Ministry of Health, Labour and Welfare regulations, and International Conference on Harmonisation Good Clinical Practice guidelines. Furthermore, the study protocol was approved by all relevant institutional review boards/ethics committees, and patients were required to provide written informed consent prior to entering the trial. The study was registered with http://clinicaltrials.gov under study number NCT00603642.

During the 3-week screening phase and the remainder of the study, platelet counts were assessed every 7 days. Eligibility criteria consisted of: ITP diagnosed at least 6 months before the initial screening visit, ≥1 previous treatment for ITP, the mean of 3 scheduled platelet counts (i.e., at weeks -3, -2, and -1 prior to study entry) had to be $\leq 30 \times 10^9$/L (with no individual count $>35 \times 10^9$/L), Japanese race, age ≥20 years, and Eastern Cooperative Oncology Group (ECOG) performance status of 0–2. If patients were found to be *H. pylori* positive, they had to have completed at least 1 course of *H. pylori* eradication therapy at least 12 weeks before the first screening visit. Furthermore, patients were required to have a hemoglobin value of ≥10 g/dL, a serum creatinine concentration of ≤2 mg/dL, and either total bilirubin ≤1.5 times the upper limit of normal (ULN) or alanine aminotransferase and aspartate aminotransferase ≤3 times ULN. Patients who received concomitant treatment with oral corticosteroids, azathioprine, and/or danazol administered at constant dose and schedule from at least 4 weeks prior to the first screening visit were permitted into the study. However, patients who had received any other drug (including immunoglobulins) to increase platelet counts within 2 weeks before screening were excluded, as were those who received hematopoietic growth factors, anti-malignancy agents, or any monoclonal antibodies within 4, 8, or 14 weeks of screening, respectively. Other key exclusion criteria included: splenectomy within 12 weeks before the first screening visit, history of bone marrow stem cell disorder or abnormal bone marrow findings (other than those typical of ITP), any active malignancy, arterial thrombosis, and a history of venous thrombosis necessitating anticoagulation therapy.

2.2 Interventions

Romiplostim was provided by the study sponsor in 5-mL glass vials as a sterile, white, lyophilized powder containing a protein concentration of 0.5 mg/mL in 10 mM histidine, 4% mannitol, 2% sucrose, and 0.004% polysorbate 20, at pH 5 when reconstituted with 1.2 mL of sterile water. Placebo was supplied in identical vials.

After the initial screening period, eligible patients were randomized to either romiplostim or placebo in a 2:1 ratio stratified by splenectomy status. The randomization procedure was conducted centrally by Transcosmos Inc. (Tokyo, Japan) using a pre-prepared randomization list and communication via fax. Treatment was administered in a blinded fashion as a subcutaneous injection on the same day as the assessment of weekly platelet response and was continued for a total of 12 weeks, with a starting dose of 3 μg/kg once weekly. Dose adjustment was permitted up to a maximum of 10 μg/kg once weekly to achieve a platelet count within the target range of ≥ 50 to $\leq 200 \times 10^9$/L. The dose was increased or decreased by 1 μg/kg after 2 consecutive weeks of platelet counts within a range of ≥10 to $<50 \times 10^9$/L or >200 to $\leq 400 \times 10^9$/L, respectively. For these patients, the dosage could be increased or decreased every 2 weeks. For patients with platelet counts of $<10 \times 10^9$/L, dosages were to be increased by 1 μg/kg every week. For platelet counts over 400×10^9/L, the romiplostim dose was held until platelet counts were under 400×10^9/L, after which the dose was decreased by 1 μg/kg every week. If a dosage reduction was required at a dose of 1 μg/kg, treatment administration was to be withheld until platelet count fell to $<50 \times 10^9$/L.

Throughout the study, rescue medication was permitted for severe bleeding or if the investigator believed that the patient was at immediate risk of bleeding. Rescue medication was defined as any medication administered for the intended purpose of raising platelet counts; permitted were intravenous immunoglobulin, platelet transfusions, corticosteroids, and an increase in dose or frequency of a concomitant oral corticosteroid, azathioprine, and/or danazol. Patients who required rescue medications during the treatment period were to continue receiving randomized therapy. After cessation of treatment following 12 weeks of therapy, patients' weekly platelet counts were monitored for an additional 12 weeks or until their platelet count dropped to $\leq 50 \times 10^9$/L, whichever occurred first.

2.3 Study end points

All end points were defined prospectively and were assessed in the full analysis set, i.e., all patients who received at least one dose of the investigational product. The primary efficacy end point was the number of weeks with platelet response, i.e., a platelet count of $\geq 50 \times 10^9$/L; platelet counts within 4 weeks following rescue medication use were not included as responses. Secondary end points included the proportion of patients with an increase of platelet count $\geq 20 \times 10^9$/L from baseline, the change from baseline in the mean of the last 4 platelet counts during weeks 2–13, the number of weeks with platelet

counts in the target range of ≥50 to ≤200 × 10^9/L during weeks 2–13, and the incidence of rescue medication use.

Assessment of bleeding symptoms, physical examination, vital sign assessment, hematology and blood chemistry tests, and recording of adverse events were performed at regular intervals throughout the study. The presence of romiplostim and TPO antibodies was assessed from blood samples obtained at week 1 pre-dose and at the end-of-treatment visit (i.e., 1 week after the scheduled visit for the last administration of the investigational product), using a previously described method [35].

2.4 Statistical analysis

Approximately, 30 patients were planned for randomization. This sample size was conservatively estimated to provide ≥90% power for a comparison of the primary end point between treatment arms using a Wilcoxon rank sum test at a 2-sided significance level of 0.05. Proportions of patients with an increase of platelet count ≥20 × 10^9/L from baseline and with rescue medication use were compared by Fisher's exact test. The changes from baseline in the mean of the last 4 platelet counts were compared by analysis of covariance using baseline platelet count as a covariate. Other prospectively planned analyses were summary statistics exploring the influence of splenectomy status and concomitant ITP therapy on the primary and secondary end points, as well as assessing median times to first platelet count of <50 × 10^9/L or the need for rescue medication after treatment cessation using a Kaplan–Meier estimate; the respective 95% CI was calculated using the Brookmeyer and Crowley method [36].

3 Results

3.1 Patient characteristics

A total of 34 patients were eligible for inclusion into the study, of which 22 were randomized to romiplostim and 12 to placebo. All participants completed the study through week 12 (Fig. 1). All patients had previously received corticosteroids for prior ITP treatment. Among the overall study population, patients had received a median of 4 (1–19) prior ITP therapies, 15 patients (44.1%) had previously undergone a splenectomy, and 23 (67.6%) were receiving concomitant ITP therapy at baseline. Of note, twice as many patients in the romiplostim group had undergone prior *H. pylori* eradication therapy. More patients in the placebo group were female and receiving concomitant ITP therapy. Otherwise, both groups were well matched for baseline demographics and clinical characteristics, including baseline platelet counts (Table 1).

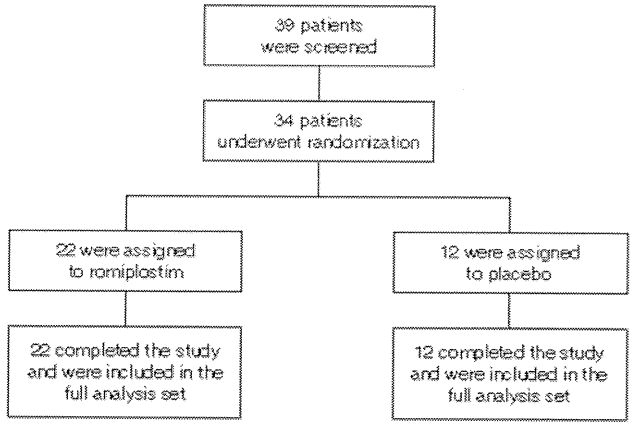


Fig. 1 Patient disposition throughout the study

The mean treatment duration was 12.0 (±0.1) weeks in the romiplostim group and 11.9 (±0.3) weeks in the placebo group. The dose for the romiplostim group increased slightly from the protocol-specified starting dose of 3 μg/kg to a mean of 3.2 μg/kg at week 2 and then decreased to a range of 2.1–2.8 μg/kg from weeks 3 to 12, whereas the mean virtual dose for the placebo group increased steadily throughout the study to a maximum of 8.7 μg/kg at week 11 (Fig. 2). Doses of romiplostim were comparable regardless of splenectomy and concomitant ITP therapy status.

3.2 Efficacy

Romiplostim was superior ($p < 0.0001$) to placebo in the primary end point of weeks of response, with a median duration of platelet response (lower quartile, upper quartile) of 11 weeks (9, 12) in the romiplostim group and 0 weeks (0, 0) in the placebo group. In patients treated with romiplostim, results were similar in splenectomized and non-splenectomized patients (median 11.0 vs. 10.5 weeks) and in patients with or without concomitant ITP therapy at baseline (median 11.0 vs. 10.0 weeks). Platelet responses could be observed in the romiplostim group as early as 1 week after the first administration of the study drug, and the response rate remained stable throughout the treatment period (Fig. 3a). Of note, splenectomy status did not appear to have an effect on weekly platelet response in either treatment arm (Fig. 3b). After week 7, treatment response appeared to be slightly better in romiplostim-treated patients administered concomitant ITP therapy ($n = 13$) than in patients who received romiplostim only ($n = 9$); however, the patient numbers in each of these subpopulations were relatively small, so this apparent difference may be due to random variation in the sample rather than a true treatment effect (Fig. 3c). The mean romiplostim dose immediately prior to the first weekly platelet response was 3.2 (±0.4) μg/kg regardless of splenectomy status and

Table 1 Patient demographics and baseline characteristics

Patient baseline characteristic	Romiplostim ($N = 22$)	Placebo ($N = 12$)
Gender, *n* (%)		
Female	14 (63.6%)	10 (83.3%)
Male	8 (36.4%)	2 (16.7%)
Age, mean (SD)	58.5 (±12.6) years	47.6 (±13.4) years
Race, *n* (%)		
Japanese	22 (100%)	12 (100%)
Weight, mean (SD)	58.3 (±11.4) kg	58.2 (±12.6) kg
ECOG performance status, *n* (%)		
0	17 (77.3%)	11 (91.7%)
1	5 (22.7%)	1 (8.3%)
Time since ITP diagnosis, mean (SD)	9.7 (±10.4) years	7.6 (±5.9) years
Platelet count, mean (SD)	18.4 (±8.3) $\times 10^9$/L	15.8 (±.6) $\times 10^9$/L
Concomitant ITP therapy, *n* (%)		
Yes	13 (59.1%)	10 (83.3%)
No	9 (40.9%)	2 (16.7%)
Splenectomy status		
Yes, *n* (%)	10 (45.5%)	5 (41.7%)
No, *n* (%)	12 (54.5%)	7 (58.3%)
Time since splenectomy, mean (SD)	8.1 (±6.8) years	10.6 (±6.9) years
Number of prior ITP treatments, median (range)	4 (1, 19)	4 (1, 7)
ITP treatment history, *n* (%)		
Corticosteroid	22 (100.0%)	12 (100.0%)
IV immunoglobulin	11 (50.0%)	8 (66.7%)
H. pylori eradication therapy	11 (50.0%)	3 (25.0%)
Azathioprine	5 (22.7%)	4 (33.3%)
Danazol	3 (13.6%)	2 (16.7%)
Cyclophosphamide	4 (18.2%)	0 (0.0%)
Rituximab	3 (13.6%)	0 (0.0%)
Vincristine/vinblastine	2 (9.1%)	0 (0.0%)
Other	10 (45.5%)	7 (58.3%)

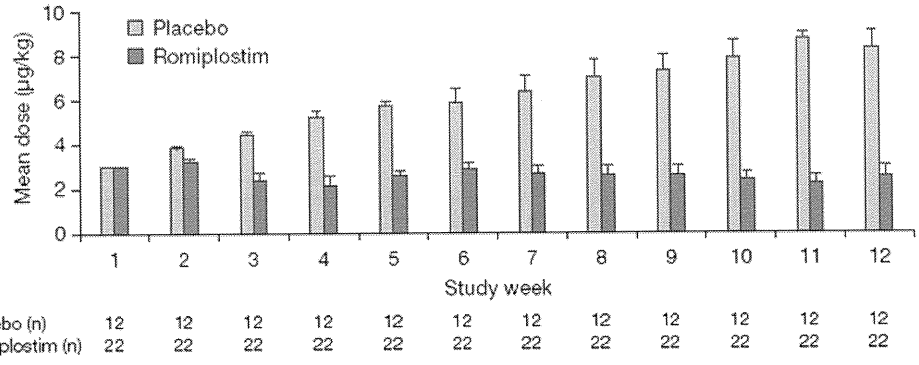


Fig. 2 Mean (+standard error) weekly doses of romiplostim or placebo and number of patients evaluated at each time point

concomitant ITP treatment. To address the concern that the imbalance in prior *H. pylori* eradication therapy between the 2 treatment groups may have influenced the observed treatment difference, a post-hoc analysis of platelet response stratified by *H. pylori* status was conducted. After stratification according to prior *H. pylori* eradication therapy, romiplostim remained superior to placebo ($p < 0.0001$).

Romiplostim was also superior to placebo in all secondary efficacy end points, except the proportion of patients requiring rescue medications, the difference of

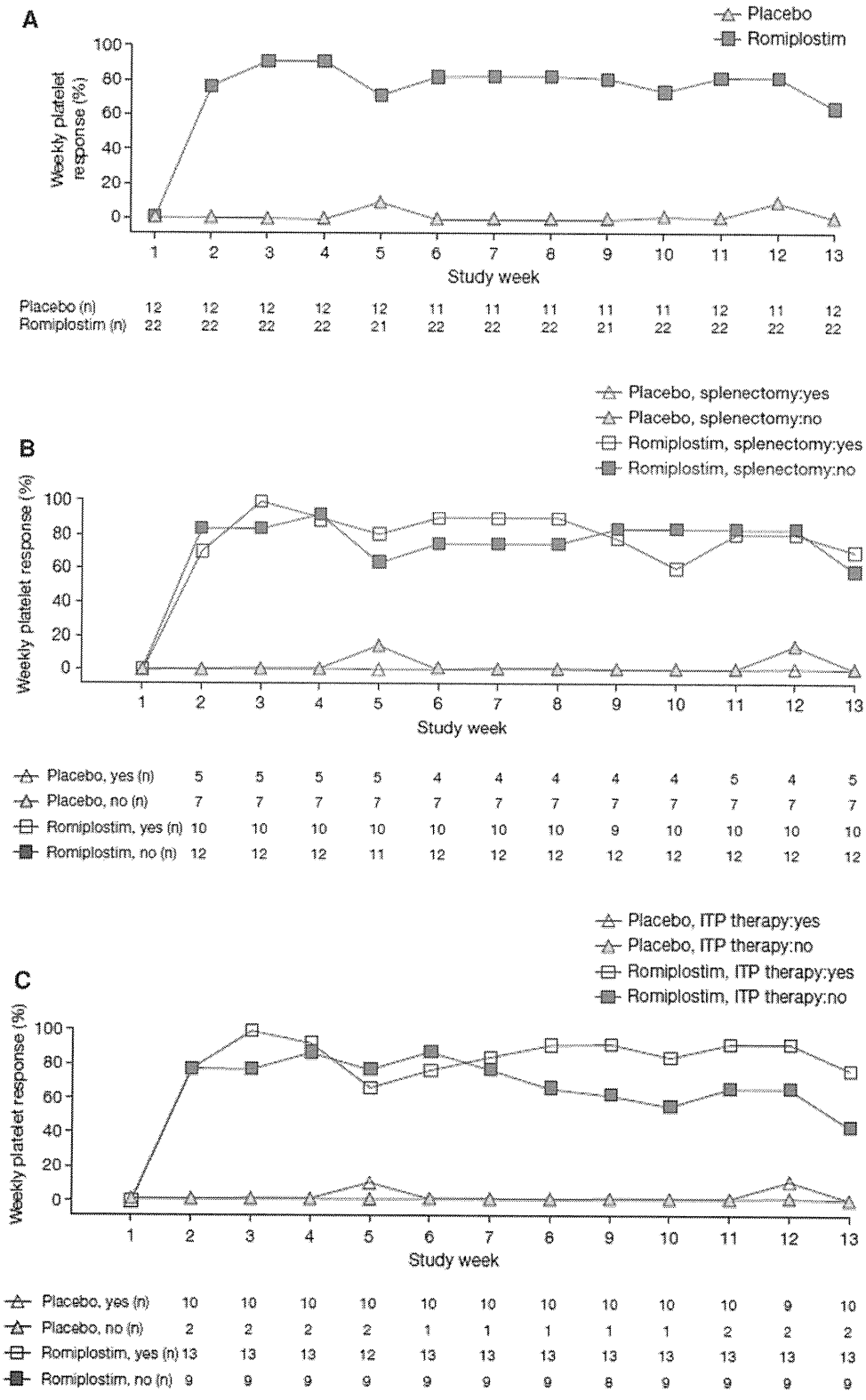


Fig. 3 Weekly platelet response in patients treated with romiplostim or placebo (**a**) and by splenectomy status (**b**) as well as baseline concomitant ITP therapy (**c**)

which was not statistically significant. Detailed results and comparisons for all efficacy end points are listed in Table 2. The mean weekly platelet count was significantly higher for patients given romiplostim than those receiving placebo at all times (Fig. 4). The number of patients whose highest platelet count was $\geq 50 \times 10^9$/L and had doubled from baseline was 21/22 (95.5%) in the romiplostim group and 1/12 (8.3%) in the placebo group. After cessation of

Table 2 Primary and secondary efficacy outcomes in patients treated with romiplostim or placebo

	Romiplostim ($N = 22$)	Placebo ($N = 12$)	p value
Primary end point			
Weekly platelet response: platelet count of $\geq 50 \times 10^9$/L, median (Q1, Q3)	11.0 (9, 12) weeks	0.0 (0, 0) weeks	<0.0001
Secondary end points			
Proportion of patients with increase of platelet count $\geq 20 \times 10^9$/L from baseline, incidence rate, n (%)	21 (96%)	3 (25%)	<0.0001
Change from baseline in mean of last 4 platelet counts during weeks 2–13, mean (SD)	110 (89) $\times 10^9$/L	2 (7) $\times 10^9$/L	0.0003
Number of weeks with platelet counts in target range of ≥ 50 to $\leq 200 \times 10^9$/L, median (Q1, Q3)	7 (3, 9) weeks	0 (0, 0) weeks	<0.0001
Proportion of patients requiring rescue medications, incidence rate, n (%)	2 (9%)	2 (17%)	0.6015

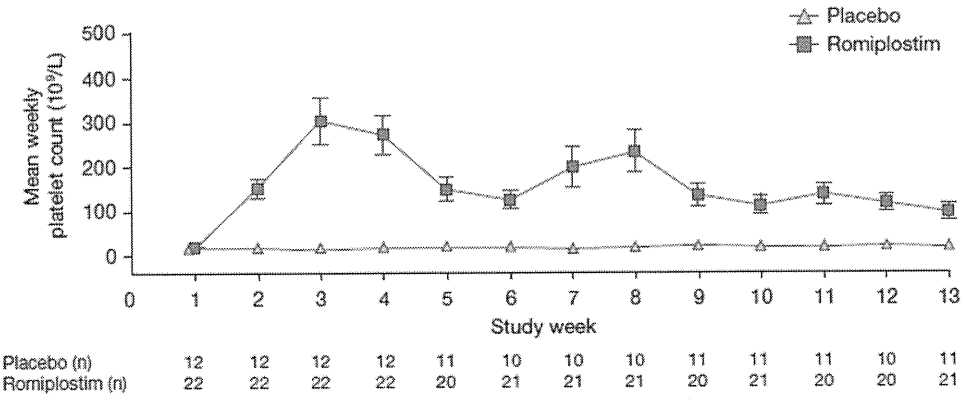


Fig. 4 Mean (±standard error) weekly platelet count and number of patients evaluated at each time point

romiplostim treatment (i.e., from week 13 onwards), the median time to a first platelet count of $<50 \times 10^9$/L or to receiving rescue medication was 1.0 weeks (95% CI 1.0, 2.0; assessed by Kaplan–Meier method). Of note, among romiplostim-treated patients, platelet counts after the 12-week treatment period remained above 50×10^9/L for 8 weeks in one study participant and for 12 weeks in another. Only 1 patient treated with romiplostim did not achieve a treatment response. This patient exhibited severe thrombocytopenia at baseline (5×10^9/L) and platelet count increased during the course of romiplostim therapy to a maximum of 19×10^9/L (week 11). Furthermore, this patient had bleeding symptoms (i.e., purpura/petechiae and oral bleeding) present at baseline, which resolved after initiation of romiplostim therapy and did not recur throughout the study.

An improvement in the occurrence of bleeding symptoms (i.e., purpura/petechiae, epistaxis, oral bleeding, menorrhagia, bruising, intracranial bleeding, gastrointestinal bleeding, and/or other bleeding symptoms) was observed in romiplostim-treated patients, including after the 12-week treatment period. The incidence of any of these bleeding symptoms in the placebo group was 83.3% at baseline and week 5, 100% at week 9, and 83.3% at week 13. Romiplostim decreased the incidence of bleeding symptoms from 63.6% at baseline to 31.8% at weeks 5 and 9, and 36.4% at week 13. During the 12-week follow-up period, 100% of placebo and 72.7% of romiplostim patients had bleeding symptoms.

3.3 Safety

Similar proportions of patients in both treatment groups (91.7% placebo, 90.9% romiplostim) experienced adverse events from any cause. Incidences of serious adverse events (8.3% placebo, 9.1% romiplostim) and adverse events that were graded 3 (severe) in severity (8.3% placebo, 9.1% romiplostim) were also similar. Adverse events that occurred in at least 10% of patients in either treatment group are shown in Table 3. Adverse events that were recorded with >5% higher frequency in the romiplostim group than in the placebo group were nasopharyngitis (41 vs. 17%), headache (32 vs. 17%), peripheral edema (18 vs. 0%), back pain (14 vs. 0%), pain in the extremities (14 vs. 0%), nephrocalcinosis (9 vs. 0%), thermal burn injury (9

Table 3 Adverse events occurring in at least 10% of patients in either treatment group

Adverse event, *n* (%)	Romiplostim ($N = 22$)	Placebo ($N = 12$)
Nasopharyngitis	9 (41%)	2 (17%)
Headache	7 (32%)	2 (17%)
Peripheral edema	4 (18%)	0 (0%)
Back pain	3 (14%)	0 (0%)
Pain in extremity	3 (14%)	0 (0%)
Malaise	1 (5%)	2 (17%)
Contusion	0 (0%)	2 (17%)

vs. 0%), thrombocytopenia (9 vs. 0%), and fatigue (9 vs. 0%). Most of these events had resolved by the end of the study, except for 2 events of back pain, all 2 events of nephrocalcinosis, 2 events of thrombocytopenia, and 1 event of fatigue. In the romiplostim group, 3 events of thrombocytopenia occurred after cessation of dosing: 1 in an 81 year-old female, first observed on day 92, ongoing at the study end, and not judged as treatment related, and 2 out of 3 observed in a 78 year-old male, in whom the final event of thrombocytopenia also occurred on day 92, remained ongoing at the study end, was not judged as treatment related, and was judged as serious. In both of these patients, platelet counts fell below those recorded at baseline. Significant (≥grade 3) bleeding events occurred in 1 patient in the romiplostim group (subarachnoid hemorrhage during week 3; week 3 platelet count was 120×10^9/L) and 1 patient in the placebo group (subarachnoid hemorrhage, cerebral hemorrhage, and gastrointestinal hemorrhage during week 10; no platelet measure taken that week, but week 11 platelet count was 23×10^9/L). There were no adverse events of bone marrow reticulin or thrombosis, and no detection of neutralizing antibodies to romiplostim or TPO.

Adverse events considered to be treatment related by the investigator also occurred in similar proportions of patients in the two treatment groups (33.3% placebo, 40.9% romiplostim); no serious treatment-related adverse events were reported. Three treatment-related adverse events were observed with a >5% higher frequency in the romiplostim group than in the placebo group. These were headache (23 vs. 8%), fatigue (9 vs. 0%), and pain in extremity (9 vs. 0%). All treatment-related events were considered mild in severity and no treatment-related event was serious. Two patients (9%) in the romiplostim group reported a total of 2 serious adverse events (thrombocytopenia and subarachnoid hemorrhage), and 1 patient (8%) in the placebo group reported a total of 3 serious adverse events (gastrointestinal hemorrhage, cerebral hemorrhage, and subarachnoid hemorrhage). Of note, no thrombotic or thromboembolic events were reported in this study. One subject in the placebo group discontinued investigational product temporarily due to moderate depression. No patients died during the study, within 30 days after the end of the treatment phase, or at the end of the study, and none withdrew from the study due to an adverse event or for any other reason.

4 Discussion

In this randomized, blinded, phase 3 trial, romiplostim at a starting dose of 3 μg/kg significantly increased and maintained platelet counts and was well tolerated in Japanese patients with ITP. This study was similar in design to previous key romiplostim trials, but with a shorter treatment duration and a somewhat higher starting dose [34, 37]. Romiplostim was superior to placebo in the primary and most secondary efficacy end points. The only secondary end point that was not statistically different between treatment groups was the use of rescue medication, which was relatively low compared to previous trials with the TPO-receptor agonists [34, 38]. Conversely, administration of concomitant ITP therapy was more than twice as high as in other phase 3 studies of romiplostim [34], and was more frequent in the placebo group. In Japan, the administration of rescue medication is typically only considered for patients with clinically important bleeding episodes (e.g., oral or severe bleeding; or those with severe thrombocytopenia [1]), while this study tended to enroll clinically stable patients with chronic ITP. Only one patient in the romiplostim arm was judged as a non-responder; of note, this individual had severe baseline thrombocytopenia and romiplostim did provide some clinical benefit (i.e., a slight increase in platelet counts and resolution of bleeding symptoms) over the course of the study period.

The safety profile of romiplostim in Japanese patients was also consistent with that seen in non-Japanese populations. There were no deaths during the course of the study, no treatment discontinuation with romiplostim, and no signs that neutralizing antibodies to either TPO or romiplostim had developed in any of the study participants. While bone marrow reticulin has been detected in some patients receiving TPO-receptor agonists [39–41], this was not observed among patients in this study. Some patients experienced thrombocytopenia after cessation of romiplostim administration; worsening thrombocytopenia is a known risk after discontinuation of therapy with romiplostim and TPO-receptor agonists in general [11]. No clinically important safety concerns were identified. Of note, the great majority of patients in this study had a clinically significant response to romiplostim as second-line ITP therapy. Furthermore, response to romiplostim appears to compare well to that achieved with other second-line therapy options, which have widely varying (10–85%) but

generally low response rates. There is currently no preference for a particular second-line therapy in this setting [1, 11]. Based on these overall observations, romiplostim appears to be a potentially useful treatment option in Japanese patients with ITP.

Racial groups exhibit potential differences in terms of drug metabolism, disease presentation, treatment response, and adverse-event profiles across a range of different disease types [29, 30]. For instance, a population pharmacokinetic study of the TPO-receptor agonist eltrombopag suggested that this agent should be initiated at lower doses in East-Asian ITP patients [42, 43]. It was therefore of interest to evaluate the efficacy and safety of romiplostim in Japanese patients in a randomized, controlled, phase 3 trial, using a study design similar to phase 3 trials conducted in largely non-Asian patient populations [34], thus allowing for a meaningful comparison between Japanese and non-Japanese patients. This study differed from previous key romiplostim trials only in that there was a shorter treatment duration and a higher starting dose (3 vs. 1 μg/kg) [34, 37]. However, the recent randomized phase 3 trial demonstrating superiority of romiplostim over standard of care in the treatment of chronic ITP in non-splenectomized patients used the same dose range as in this study [40]. In our study, treatment with an initial dose of 3 μg/kg yielded a very rapid therapeutic response in about 80% of patients from week 2 onwards. In comparison, past studies with a starting dose of 1 μg/kg, resulted in somewhat slower attainment of response, i.e., approximately 50% of patients in the same timeframe [34]. Although no safety concerns (e.g., thrombotic events caused by the increase in platelet counts) were identified with the higher starting dose, platelet counts should be closely monitored and the romiplostim dose modified until stable, adequate platelet counts have been achieved.

This study was somewhat limited by its relatively short duration (i.e., a 12-week treatment period and 12-week follow-up period) and the small number of participants. The relatively small sample size, especially of the subpopulations, should be considered when evaluating the study results. For instance, random variation rather than a true treatment effect may have contributed to the slight differences observed (about half-way through the trial) in platelet responses among patients treated with romiplostim only as compared with those who received additional concomitant ITP therapy. Inclusion criteria specified that ITP be diagnosed at least 6 months prior to study entry, as per the definition of chronic ITP generally accepted at the time the trial was designed. This is shorter than the currently accepted definition of chronic ITP, which requires the disorder to have been present for a year before a diagnosis of chronic disease is made [7]. However, the long mean time since ITP diagnosis observed in both treatment groups confirms that we studied a patient population consistent with the current definitions of chronic adult ITP. The results of this phase 3 trial clearly suggest a clinical benefit of romiplostim for the second-line treatment of chronic ITP in Japanese patients, in a patient population that closely reflects that treated for refractory ITP in clinical practice. While the study duration was sufficient to evaluate the efficacy and safety of romiplostim in a Japanese population, future investigations should continue to assess the long-term use of romiplostim in this setting.

In conclusion, romiplostim was an effective, safe, and well-tolerated agent for the treatment of ITP in Japanese patients; The efficacy and safety results were largely consistent with those previously obtained in a non-Japanese patient population. The results of this study lend further support to current clinical guidelines [11] that include romiplostim as a second-line therapy option for the treatment of chronic ITP in patients with insufficient response to other treatments.

Acknowledgments This study was sponsored by Amgen Inc. The authors would like to thank all study investigators and the study coordinators, as well as Dr. Kiyoshi Kitamura (Tokyo University), who provided medical advice during the study. The authors would also like to thank Dominik Wolf, MSc (PAREXEL) and Dr. Susanna Mac (Amgen Inc.) who provided scientific writing assistance.

Conflict of interest Y.T. received speaker's honoraria from Kyowa Hakko Kirin Co. and GlaxoSmithKline; M.K. is a consultant to Novartis and Shionogi & Co., Ltd.; H.W. and R.L. are stockholders and employees of Amgen Inc. The remaining authors have no relevant conflicts of interest to report.

References

1. Kurata Y. Recent advances in the treatment of idiopathic thrombocytopenic purpura. Rinsho Ketsueki. 2007;48:355–62.
2. Chang M, Nakagawa PA, Williams SA, et al. Immune thrombocytopenic purpura (ITP) plasma and purified ITP monoclonal autoantibodies inhibit megakaryocytopoiesis in vitro. Blood. 2003;102:887–95.
3. Ijichi T, Muranishi M, Shimura K, et al. Idiopathic thrombocytopenic purpura and mononeuropathy multiplex. Acta Haematol. 2003;110:33–5.
4. Nugent D, McMillan R, Nichol JL, Slichter SJ. Pathogenesis of chronic immune thrombocytopenia: increased platelet destruction and/or decreased platelet production. Br J Haematol. 2009;146:585–96.
5. Provan D. Characteristics of immune thrombocytopenic purpura: a guide for clinical practice. Eur J Haematol Suppl. 2009;(71):8–12.
6. Pruemer J. Epidemiology, pathophysiology, and initial management of chronic immune thrombocytopenic purpura. Am J Health Syst Pharm. 2009;66:S4–10.
7. Rodeghiero F, Stasi R, Gernsheimer T, et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. Blood. 2009;113:2386–93.

8. Zhang F, Chu X, Wang L, et al. Cell-mediated lysis of autologous platelets in chronic idiopathic thrombocytopenic purpura. Eur J Haematol. 2006;76:427–31.
9. Deane S, Teuber SS, Gershwin ME. The geoepidemiology of immune thrombocytopenic purpura. Autoimmun Rev. 2010;9: A342–9.
10. Fogarty PF. Chronic immune thrombocytopenia in adults: epidemiology and clinical presentation. Hematol Oncol Clin North Am. 2009;23:1213–21.
11. Provan D, Stasi R, Newland AC, et al. International consensus report on the investigation and management of primary immune thrombocytopenia. Blood. 2010;115:168–86.
12. Stasi R, Stipa E, Masi M, et al. Long-term observation of 208 adults with chronic idiopathic thrombocytopenic purpura. Am J Med. 1995;98:436–42.
13. Arnold DM, Bernotas A, Nazi I, et al. Platelet count response to H. pylori treatment in patients with immune thrombocytopenic purpura with and without *H. pylori* infection: a systematic review. Haematologica. 2009;94:850–6.
14. Arnold DM, Stasi R. Does *Helicobacter pylori* eradication therapy result in a platelet count improvement in adults with immune thrombocytopenic purpura regardless of *H. pylori* infection? ASH evidence-based review 2008. Hematology Am Soc Hematol Educ Program. 2008;31–2.
15. Fujimura K, Kuwana M, Kurata Y, et al. Is eradication therapy useful as the first line of treatment in *Helicobacter pylori*-positive idiopathic thrombocytopenic purpura? Analysis of 207 eradicated chronic ITP cases in Japan. Int J Hematol. 2005;81:162–8.
16. Kuwana M, Ikeda Y. *Helicobacter pylori* and immune thrombocytopenic purpura: unsolved questions and controversies. Int J Hematol. 2006;84:309–15.
17. Suzuki T, Matsushima M, Masui A, et al. Effect of *Helicobacter pylori* eradication in patients with chronic idiopathic thrombocytopenic purpura—a randomized controlled trial. Am J Gastroenterol. 2005;100:1265–70.
18. Tsumoto C, Tominaga K, Okazaki H, et al. Long-term efficacy of *Helicobacter pylori* eradication in patients with idiopathic thrombocytopenic purpura: 7-year follow-up prospective study. Ann Hematol. 2009;88:789–93.
19. Abrahamson PE, Hall SA, Feudjo-Tepie M, et al. The incidence of idiopathic thrombocytopenic purpura among adults: a population-based study and literature review. Eur J Haematol. 2009; 83:83–9.
20. Schoonen WM, Kucera G, Coalson J, et al. Epidemiology of immune thrombocytopenic purpura in the General Practice Research Database. Br J Haematol. 2009;145:235–44.
21. Kurata Y, Fujimura K, Kuwana M, et al. Epidemiology of primary immune thrombocytopenia in children and adults in Japan: a population-based study and literature review. Int J Hematol. 2011;93:329–35.
22. Stasi R, Provan D. Management of immune thrombocytopenic purpura in adults. Mayo Clin Proc. 2004;79:504–22.
23. Godeau B, Michel M. Treatment of chronic immune thrombocytopenic purpura in adults. Ann Hematol. 2010;89:55–60.
24. McMillan R. Therapy for adults with refractory chronic immune thrombocytopenic purpura. Ann Intern Med. 1997;126:307–14.
25. Nomura S, Dan K, Hotta T, et al. Effects of pegylated recombinant human megakaryocyte growth and development factor in patients with idiopathic thrombocytopenic purpura. Blood. 2002; 100:728–30.
26. Rank A, Weigert O, Ostermann H. Management of chronic immune thrombocytopenic purpura: targeting insufficient megakaryopoiesis as a novel therapeutic principle. Biologics. 2010;4: 139–45.
27. Nplate (romiplostim), European Public Assessment Report. European Medicines Agency. 2009. Available at http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000942/human_med_000938.jsp&murl=menus/medicines/medicines.jsp&jsenabled=true. Accessed 16 June 2011.
28. Romiplate prescribing information. 2011. http://www.mhlw.go.jp/stf/shingi/2r985200000136yg-att/2r9852000001372i.pdf. Accessed 16 June 2011.
29. Burroughs VJ, Maxey RW, Levy RA. Racial and ethnic differences in response to medicines: towards individualized pharmaceutical treatment. J Natl Med Assoc. 2002;94:1–26.
30. Matthews HW. Racial, ethnic and gender differences in response to medicines. Drug Metabol Drug Interact. 1995;12:77–91.
31. Kumagai Y, Fujita T, Ozaki M, et al. Pharmacodynamics and pharmacokinetics of AMG 531, a thrombopoiesis-stimulating peptibody, in healthy Japanese subjects: a randomized, placebo-controlled study. J Clin Pharmacol. 2007;47:1489–97.
32. Shirasugi Y, Ando K, Hashino S, et al. A phase II, open-label, sequential-cohort, dose-escalation study of romiplostim in Japanese patients with chronic immune thrombocytopenic purpura. Int J Hematol. 2009;90:157–65.
33. Bussel JB, Kuter DJ, George JN, et al. AMG 531, a thrombopoiesis-stimulating protein, for chronic ITP. N Engl J Med. 2006;355:1672–81.
34. Kuter DJ, Bussel JB, Lyons RM, et al. Efficacy of romiplostim in patients with chronic immune thrombocytopenic purpura: a double-blind randomised controlled trial. Lancet. 2008;371: 395–403.
35. Jawa V, Hokom M, Hu Z, et al. Assessment of immunogenicity of romiplostim in clinical studies with ITP subjects. Ann Hematol 2010.
36. Brookmeyer R, Crowley J. A confidence interval for the median survival time. Biometrics. 1982;38:39–41.
37. Bussel JB, Kuter DJ, Pullarkat V, et al. Safety and efficacy of long-term treatment with romiplostim in thrombocytopenic patients with chronic ITP. Blood. 2009;113:2161–71.
38. Cheng G, Saleh MN, Marcher C, et al. Eltrombopag for management of chronic immune thrombocytopenia (RAISE): a 6-month, randomised, phase 3 study. Lancet. 2011;377:393–402.
39. Kuter DJ, Mufti GJ, Bain BJ, et al. Evaluation of bone marrow reticulin formation in chronic immune thrombocytopenia patients treated with romiplostim. Blood. 2009;114:3748–56.
40. Kuter DJ, Rummel M, Boccia R, et al. Romiplostim or standard of care in patients with immune thrombocytopenia. N Engl J Med. 2010;363:1889–99.
41. Saleh MN, Bussel JB, Meyer O, et al. Results of bone marrow examinations in patients with chronic immune thrombocytopenic purpura treated with eltrombopag [abstr]. Blood. 2009;114:1326.
42. Gibiansky E, Zhang J, Williams D, et al. Population pharmacokinetics of eltrombopag in healthy subjects and patients with chronic idiopathic thrombocytopenic purpura. J Clin Pharmacol. 2011;51:842–56.
43. Promacta (eltrombopag) tablets. US prescribing information. GlaxoSmithKline: Research Triangle Park; 2010.

Cytometry Research 22（1）：7 ～ 11，2012

特集 1

同一検体を用いた CD34 陽性細胞率の検討

米村　雄士[1)]，福吉　葉子[1)]，下山　治香[1)]，甲斐　由希美[1)]，山野　知子[1)]，内場　光浩[1)]，
日高　道弘[2)]，安東　由喜雄[1)3)]

Analysis of CD34-positive cells using the same PBSCH or BM samples

Yuji Yonemura M.D.,PhD.[1)], Yoko Fukuyoshi[1)], Haruka Shimoyama[1)], Yukimi Kai[1)], Tomoko Yamano[1)], Mitsuhiro Uchiba M.D.,PhD.[1)], Michihiro Hidaka M.D.,PhD.[2)], Yukio Ando M.D.,PhD[1)3)].

[1)] Department of Transfusion Medicine and Cell Therapy, Kumamoto University Hospital
[2)] National Hospital Organization Kumamoto Medical Center
[3)] Department of Diagnostic Medicine, Kumamoto University Hospital

抄録

当院では，1999 年から末梢血幹細胞採取（PBSCH：peripheral blood stem cells harvest）を開始し，CD34 の測定は，2002 年からベックマン・コールター社の Stem-Kit を用いて開始した。今回 2011 年 1 月～ 4 月に自施設及び熊本医療センターの PBSCH 検体及び骨髄採取検体を用いて，自施設の当日，翌日の CD34 陽性率と検査センターで翌日測定した CD34 陽性率を比較検討した。CD34 陽性率は CD34 抗体の種類やクローンの違いにより，データに差異がでてくると思われるが，今回のように，クローン 581 と 8G12 のようなクラス III の抗体であれば，陽性率は相関係数 r=0.996 と全く問題を認めなかった。またフローサイトメトリーの機種の違いや，翌日測定することも CD34 陽性率にはほとんど影響与えなかった。

Abstract

At our hospital, we started collection of peripheral blood stem cells (PBSC) in 1999, and began to examine the CD34 phenotype of the PBSC using the Stem-Kit (Beckman-Coulter) in 2002. We compared the CD34-positive rate between our data on the day of collection or on the following day and those of the inspecting company on the following day. The CD34-positive rate differed slightly depending on the type and clone of the anti-CD34 antibody used. When a class III antibody of anti-CD34 was used, such as clone 581 or 8G12 as in this study, the correlation coefficient of the CD34-positive rate between our hospital and the inspecting company was r=0.996, posing no problems.

Furthermore, there was also no influence of either the flow cytometric equipment used or of examination on the day after the harvest.

Key words : PBSC, CD34, CD45, ISHAGE, viability

はじめに

ヒト造血幹細胞の定量には，現在 CD34 陽性細胞数をフローサイトメトリーにて CD34 抗体と CD45 抗体を組み合わせた 2 カラー分析で測定を行っている。今回同一検体を用いて，ISHAGE（International Society for Hemato-therapy and Graft Engineering）のガイドラ

[1)] 熊本大学医学部附属病院輸血・細胞治療部
[2)] 国立病院機構熊本医療センター
[3)] 熊本大学医学部附属病院中央検査部

受付日：平成 23 年 11 月 24 日　受理日：平成 23 年 11 月 29 日

インに準じて[1][2][3]，自施設で当日と翌日測定したデータと検査センターにて翌日測定したデータの比較検討を行ったので報告する。

サンプル調整

2011年1月から3月にPBSCHを行った8人，11検体と骨髄採取を行った2人，2検体，計13検体を異なった機種のフローサイトメーターを用いて，違ったクローンのCD34，CD45抗体を用いて，CD34陽性率を自施設と検査センターで測定を行った（Table 1）。

自施設では，13検体すべてを採取当日に測定し，そのうち7検体は，翌日にも測定した。測定は同一検体で2回測定し，平均値をCD34陽性率とした。検査センターでは，通常どおりすべての検体を翌日に測定した。

CD34陽性率の測定結果

CD34陽性率の結果は，Table 2に示すとおりである。#10を除く，13検体中12検体で，自施設と検査センターのデータは近似の値を示し，相関係数はr=0.996であった（Fig. 1）。実際のフローサイトメトリーパターンはFig.2A~Dの如く，代表的4検体（Fig. 2A：#1, Fig. 2B：#5, Fig. 2C：#6, Fig. 2D：#10）のデータを示す。Table 2とFig. 2の自施設のデータがわずかに違うのは，Fig. 2は2回測定したものの一方のデータを示しているためである。Fig. 2A~Cはほぼ同じパターンで，同じ値であるが，viabilityに関しては，Table 3のように当日測定した検体が#2を除くすべての検体で死細胞の割合が少なく，最も数値がかけ離れていた#10において，viabilityが極端に悪く，そのためCD34陽性率が高値を示した可能性が考えられた。

Table 1

	自施設	検査センター
機器メーカー	ベックマン・コールター社	ベクトンディッキンソン社
測定機器	Cytomics Fc500	FACS Calibur
CD34	クローン581(classⅢ)(PE)	クローン8G12(classⅢ)(PE)
CD45	クローンJ3 (FITC)	クローン2D1(FITC)

Table 2

採取施設	症例	自施設		検査センター
		当日	翌日	翌日
熊本医療センター	#1. BM	1.98	1.69	1.98
	#2. BM	3.83	ND	4.07
	#3-1	0.33	ND	0.28
	#3-2	0.70	0.81	0.80
	#4-1	0.08	ND	0.07
	#4-2	0.04	ND	0.04
	#5	0.47	ND	0.54
自施設	#6	0.40	0.41	0.41
	#7	0.44	0.45	0.50
	#8-1	0.24	0.28	0.32
	#8-2	0.15	0.15	0.15
	#9	0.02	0.02	0.01
	#10	1.81	ND	2.24

ND: not done

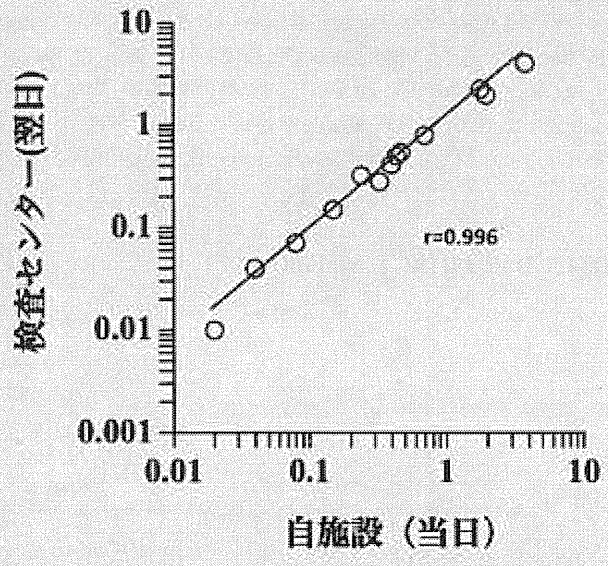


Figure 1 自施設（当日）と検査センター（翌日）で測定されたCD34陽性率の相関

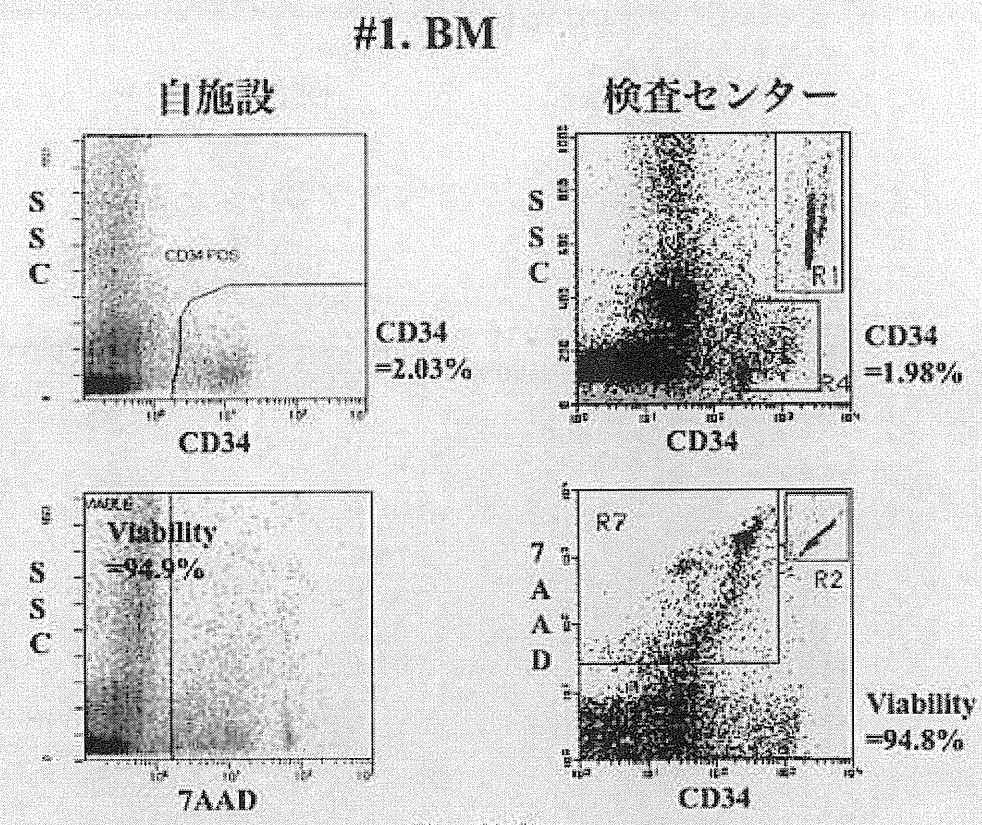


Figure 2A：#1

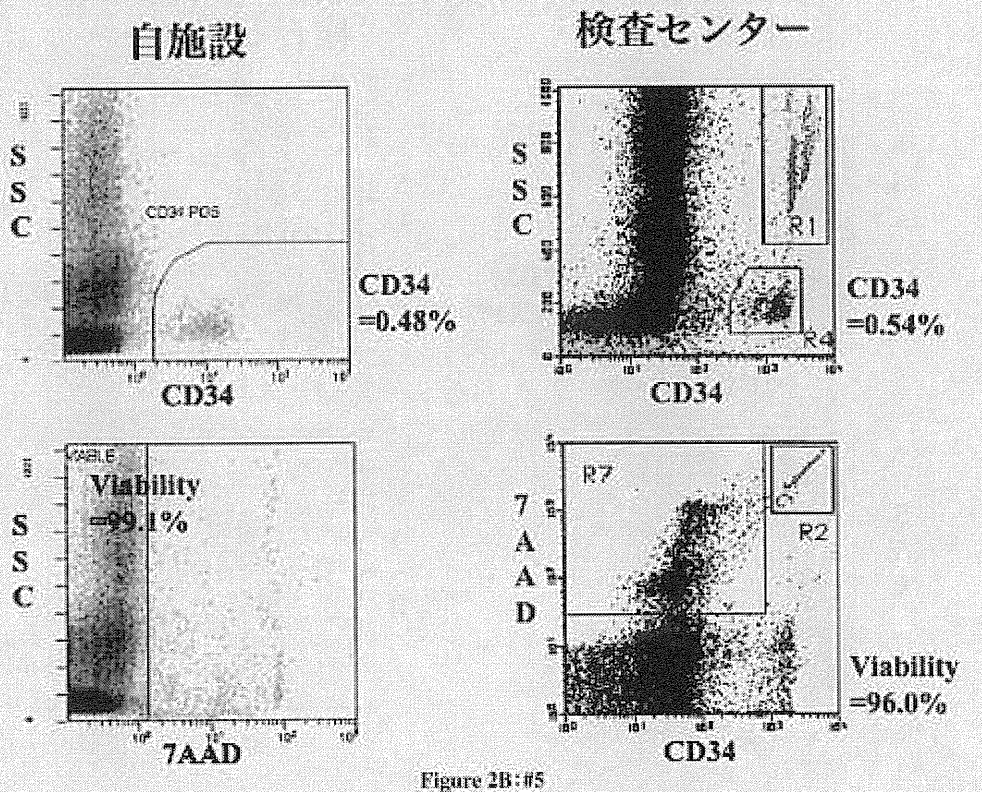


Figure 2B：#5

Figure 2 自施設（当日）と検査センター（翌日）で測定されたCD34とTAADのフローサイトメトリーパターン。